

Improvement of Cellulose Hydrolysis Process and Cost Savings

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The hydrolysis process to obtain the so-called “reducing sugars” represents the main step involved in the production of the second generation bioethanol. This product can be obtained directly from various types of green biomass, replacing the use of cereals cultivations, with obvious benefits to the environment and the economy of agricultural production. However, it is necessary to improve the hydrolysis process of the cellulose to achieve this goal. To this purpose, we applied a chemical process formerly used. The values of sugars yield were increased by about 40% with respect to the previous study. Further significant cost savings were accomplished, resulting from the recovery of the by-product, calcium sulfate, commercially known as gypsum.

Keywords: bioethanol, biomass, cellulose hydrolysis, reducing sugars

Introduction

The term biomass refers to any organic material, plankton, algae, trees, and the organic tissues of living beings. Usually, it excludes simple molecules such as water, carbon dioxide, or methane, which do not keep a record of the complex synthesis that occurs in living organisms.

Most of the biomass present on Earth is of vegetable origin. In fact, only about 10% is of animal origin. In these last years, the research is increasingly focused on studying new uses of the raw materials. The plant biomass is produced using solar energy through photosynthesis and is available in the form of forests, crops, agro-industry and agro-industrial residues, or the organic component from the collection of municipal solid waste.

Biomass is one of the most important available human resources as a renewable source of food, energy, and raw materials. The importance of its exploitation is mainly due to the net CO₂ emission reduction since this gas (known for its greenhouse effects) is absorbed through photosynthesis to produce new biomass. The plant biomass consists of compounds with high molecular weight, such as cellulose, hemicellulose, polymers of saccharide molecules, the lignin and other, relatively more simple, such as the organic and inorganic extractives. Their relative amounts vary depending on the species and age of the plant.

Cellulose is the major constituent of lignocellulosic biomass (30%-60% by weight). The establishment of hydrogen bonds between the chains leads to the formation of crystalline domains that impart strength and

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elasticity to the plant fibers. It occurs naturally in its pure state only in annual plants such as cotton and linen, while in woody plants is always accompanied by hemicellulose and lignin (see Figure 1). Hemicellulose constitutes 10%-40% of the lignocellulosic biomass and is a mixed polymer, relatively short (10-200 molecules) and branched, consisting of both six-carbon sugars (glucose, mannose, galactose) and five-carbon sugars (xylose and arabinose). Lignin is one of the main constituents of the plants since it represents 10%-30% of the mass, in dependence of the species. Its main function is to bind and cement together the various fibers to give firmness and strength to the plant, forming a protection against insects, pathogens, injury, and ultraviolet light (Gressel, Vered, Bar-Lev, Milstein, & Flowers, 1983; Wyman, 1994; Ragauskas, Williams, Davison, Britovsek, Cairney, Eckert, ..., & Tschaplinski, 2006; Lange, 2007).

Using biochemical and thermochemical conversion processes, it is possible to obtain a large variety of products from biomass, such as “green” fuels, sugar, compost, pellets, and paper pulps. Therefore, the benefits that may result from a greater exploitation of biomass are numerous, including:

- (1) New market outlets for surplus agricultural products;
- (2) Production of biodegradable materials at competitive costs;
- (3) Disposal of agro-industrial residues;
- (4) Use of local resources.

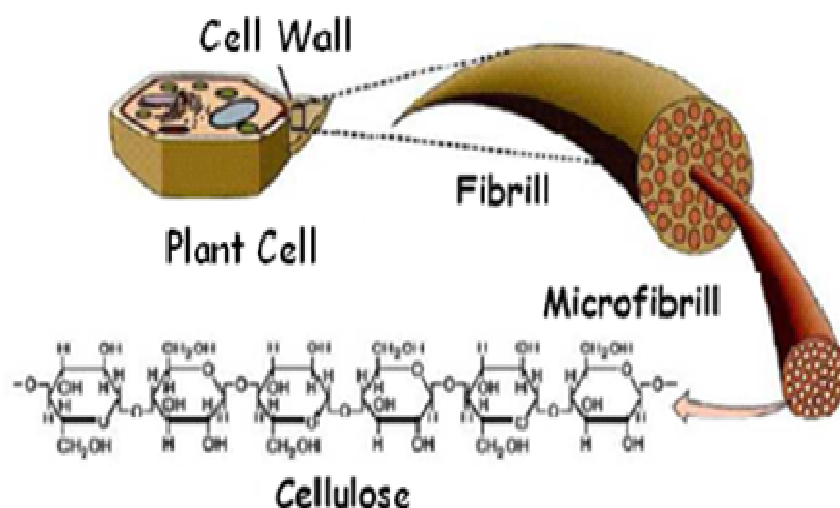


Figure 1. Structure of the cell wall.

The benefits related to biofuels are due to a lower environmental impact compared to petroleum derivatives and the use of waste materials that are not normally used (see Figures 2-3). The “ligno-cellulosic” bioethanol shows the best compromise between price, availability (agro-forestry residues), and performance enhancement of biomass energy, and improves the balance of CO₂ emissions (Tan, Culaba, & Purvis, 2004).

There are two types of production process through which bioethanol can be obtained. In the first, said first generation of ethanol, the process involves as feedstock a “sugar juice”, made from the pressing plant, high in sugar (sucrose), such as sugar cane, sugar beet, sweet sorghum.

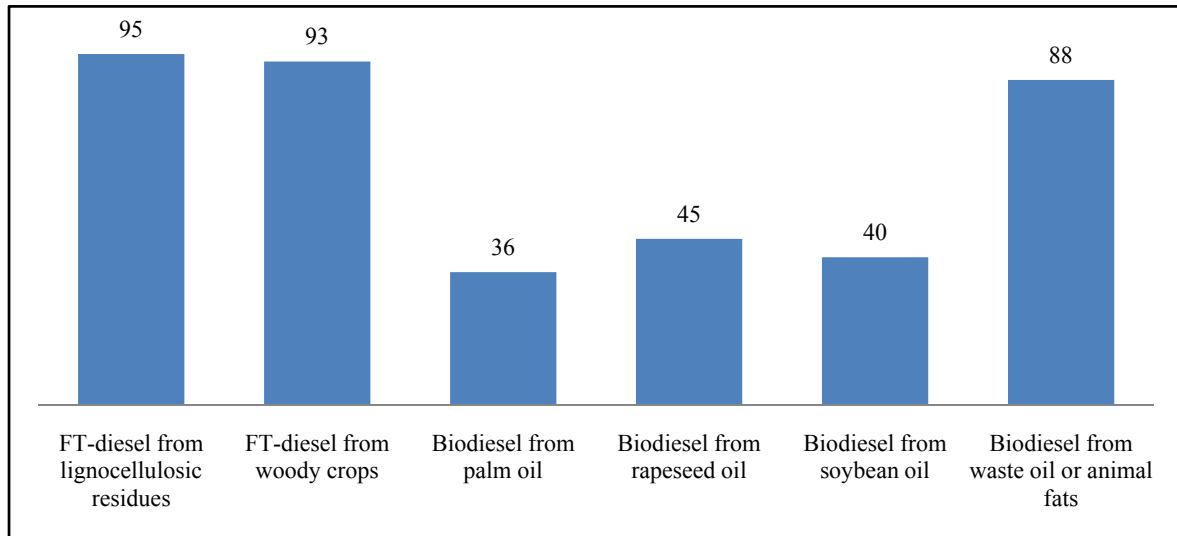


Figure 2. Estimated reduction (%) in greenhouse gas emissions for some production chains of biofuels compared with diesel oil (ENEA, n.d.).

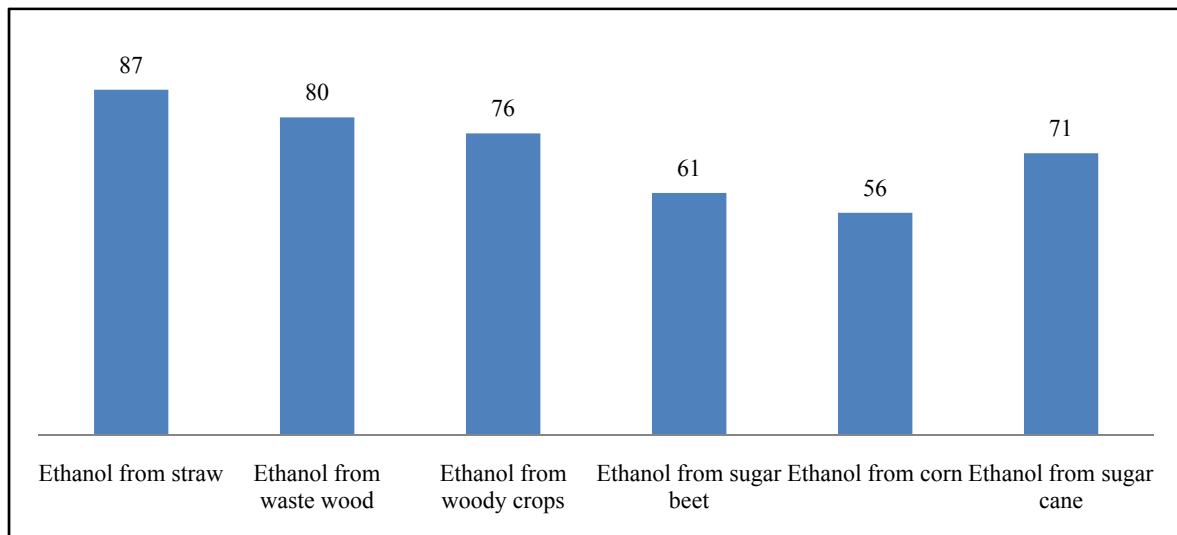


Figure 3. Estimated reduction (%) in greenhouse gas emissions for some production chains of biofuels compared with gasoline (ENEA, n.d.).

In the second type of process, called second-generation ethanol, the significant difference lies in the origin of sugar that will ferment. In fact, glucose is not only derived from starch or sugar from the plant itself, but rather is generated by chemical or enzymatic hydrolysis of cellulose and hemicellulose, the main constituent of plant fibers (Bartacek, Zabranska, & Lens, 2007; Lynd, Laser, Bransby, Dale, Davison, Hamilton, ..., & Wyman, 2008). The production process of ethanol from biomass is divided into five stages (Sun & Cheng, 2002; Waltz, 2008):

- (1) Pre-treatment;
- (2) Detoxification;
- (3) Hydrolysis;
- (4) Fermentation;
- (5) Distillation.

In the natural form, the lignocellulosic material is very structured and cannot be converted directly into the desired product. To perform the conversion, for example, sugars or biofuels, it is necessary to facilitate the breaking of chemical bonds between the various components of the biomass in order to determine a better hydrolysis. Therefore, for an effective conversion, preliminary treatment is always needed whose main objectives are:

- (1) To minimize the loss of carbohydrates;
- (2) To maximize the recovery of valuable by-products, such as lignin and hemicelluloses;
- (3) To minimize initial and operational costs;
- (4) To maximize the hydrolysis yields;
- (5) Absence of toxic by-products, inhibitors of subsequent hydrolysis.

Preliminary Studies

The method adopted for the chemical hydrolysis has been recently proposed by different authors (Galbe & Zacchi, 2007; Waltz, 2008). It involves the below-mentioned three phases (the reagent quantities were defined for the treatment of an initial amount of 2.0 grams of dried raw material):

(1) Pre-treatment performed with 50 ml of 15% caustic soda solution and 100 ml of water at 120°C, for 60 minutes. A side effect of the pre-treatment is the formation of by-products (compounds obtained by degradation of the furan, low molecular weight organic acids, phenolic compounds) during the partial degradation of the cell wall components, which inhibit the fermentative activity of the microorganisms;

(2) Detoxification allows the removal of the inhibitors: by varying the pH with $\text{Ca}(\text{OH})_2$ and H_2SO_4 , the sample after the pre-treatment is subjected to the following phases:

(a) Phase 1: pH 9, 60°C for 30 minutes;

(b) Phase 2: pH 6, 30°C for 30 minutes. Sulfuric acid action increases the effects of the hydrolytic treatment, by means of a further fragmentation of the biomass polymers into soluble oligomers and monomers. Its use is useful at low temperatures, since it increases the solubilization of biomass, as in the case of xylose.

(3) Hydrolysis with 150 ml of 30% H_2SO_4 solution at 100°C for one hour. In the reactor the ratio of acid: cellulose and hemicellulose is 1.25:1. The gel formed is then pressed to separate the fluid acid/sugar from the solid residue (lignin), which precipitates. The cellulose, once hydrolyzed, releases glucose molecules while hemicellulose releases xylose.

It is well known that the cellulose hydrolysis to reducing sugars is an expensive process from the point of view of energy consumption, that it is difficult to realize, and that it occurs with low yields. Therefore, our research concerns the optimization of the chemical process in order to obtain a better yield. In a previous study, by adopting the cited method, we utilized a single reactor, in which the production chain occurred consecutively (Calabrese, Quarantotto, & Stancher, 2011).

A laboratory system, capable of controlling the main chemical and chemical-physical variables, such as temperature, pressure, and pH, was employed. The system was based on a shaker (so that the biomass was always in contact with the air) in which the pre-treatment, detoxification, and hydrolysis occurred consecutively. After finishing the hydrolysis process, the acid solution containing the sugars was filtered through activated charcoal, by using a vacuum pump, neutralized with NaOH and brought to a final defined volume. On this solution, the assay of reducing sugars was carried out, by using the bicinchoninic acid method.

Under the cited conditions, we were able to obtain a yield of 4.4% of the reducing sugars calculated with respect to the total amount of the raw material. Anyway, it is to note that this yield, if calculated regarding the theoretical cellulose content of above 40%, in raw materials, increases at about 11%.

The main aim of this work was to study the optimization of the biomass treatment in order to improve the recovery of the fractions and to proceed to their use as substrates for the production of sugars. The economic difficulties of the chemical hydrolysis process consist essentially in:

(1) Low yield: As stated above, by following the reported procedure;

(2) Large amount of by-products: Under the cited conditions by using 2.0 g of dried raw material, we have previously obtained 88 mg of reducing free sugars but at the same time, we get more than 100 grams of salts, essentially a mixture of sodium and calcium sulphate coming from the various acid-base treatments along the different steps of the reaction. This salts precipitate consecutively in the same reaction environment;

(3) Relatively high costs of the reagents: Sulfuric acid, sodium hydroxide, and calcium hydroxide are the main reagents used;

(4) Little or no value of the by-products, especially if compared with the costs of the corresponding pure reagents. This circumstance is due to the fact that the sequence of the different steps in the same reactor does not allow separating the various salts formed during the whole reaction, so that at the end a mixture will be obtained.

In order to reduce these negative factors and after various preliminary attempts, we decided to proceed as follows.

Pre-treatment

We substituted the sodium hydroxide with calcium hydroxide in order to eliminate one of the two bases. Our aim was to obtain, at the end of the process, only calcium sulphate as by-product, in other words, to obtain a relatively pure by-product with potential possibilities of re-employment (calcium sulphate is essentially gypsum). Furthermore, this substitution permits to produce fewer by-products in the first step of the detoxification sequent, because of the stoichiometry of the neutralization reaction (from pH 9 to pH 6). In fact, the neutralization of 1 mole of sulfuric acids (98 grams) leads to 136 g of salt (calcium sulphate) if performed with calcium hydroxide, and to 232 grams of salt (sodium sulphate) if performed with sodium hydroxide. The less by-product quantity gives another potential advantage in itself. In fact, we noted that a certain amount of sugar molecules may remain adsorbed in the solid phase, during the salt precipitation, so that we can suppose that even the less quantity of formed salt can contribute to enhancing the yield of reducing sugars.

Detoxification

We noted that the detoxification phase can efficiently occur, even by introducing a certain dilution of the reagents. Furthermore, it seemed that the use of diluted solution (about 10%) of sulfuric acid and calcium hydroxide, by employing larger water volumes, can also slow down or even in some way inhibit the salt precipitation.

Hydrolysis

As stated below, the elongation of the hydrolysis time does not seem to influence significantly the reaction yield. In addition, the introduction of a subsequent step, consisting in the extraction from the solid phase by-product of sugar molecules co-precipitated during the hydrolysis process of the cellulose, appeared to be very interesting. As reported, for this purpose, we have tested different solvents; the most efficient solvent appeared to be the methanol.

Materials and Methods

Sample Preparation

The sample for the hydrolysis has been prepared starting from the vegetable matrix in its natural state (a mixture of lawn grass clover (*Trifolium* L., 1753) and grasses (*Cynodon dactylon* (L.) Pers, 1805)). To this purpose, approximately 500 g of material were subjected to drying in a stove at 80°C for three hours, and then ground and sieved with a particle size ≤ 1.00 mm. Afterwards, an aliquot of 2 g of dry matter was used for the subsequent steps. All the reagents used were of Lab. purity grade; deionized water was used during the different phases.

Moisture Determination

The residual moisture of the sample, prepared as mentioned above, was determined by weight loss as a result of heating of 2.0 g of matter in a stove at 105°C, according to the official methods. In these conditions, the average value, expressed as an average of six tests, was $5.0\% \pm 0.5$. A further aliquot of 2.0 g was subjected to chemical hydrolysis process.

Recovery of Reducing Sugars From the Salt

An aliquot of 30 grams of salt, formed as by product, after filtration from the liquid phase, were washed overnight, with about 200 ml of methanol in a pyrex flask at room temperature, with a water refrigerant, under magnetic stirring. Subsequently, the liquid was filtered, put in a “Rotavapor” flask and dried under a vacuum water pump (80% of methanol was recovered therefrom). After that, the flask was washed with water and the solution was transferred in an Erlenmayer flask and taken up to 100 ml. An aliquot of this solution was then used for the test with the bicinchoninic acid.

The Testing Method for Reducing Sugars

The method is based on the fact that under alkaline conditions, copper Cu^{2+} ions form a complex with the reducing ends of sugars molecules and then are reduced to Cu^+ . Under these conditions, a purple color complex is formed, whose intensity is proportional to the amount of sugar present. The color intensity is determined by measuring the absorbance in a spectrophotometer at 562 nm. The concentration values are then extrapolated from a calibration curve previously constructed using samples of known concentration of glucose, in the range of 2.5-100 mg/l. The reactive consists of two parts:

- (1) A: 4.5 g of bicinchoninic acid and 215 g of anhydrous sodium carbonate in 3.45 l H_2O ;
- (2) B: 3.7 g of aspartic acid and 5 g of sodium carbonate in 100 ml H_2O ; 1 g of copper sulfate in 150 ml H_2O .

The final reagent is prepared by mixing 23 parts of A and 1 of B. In the cuvette for the absorbance measure, reagent and sample were at a ratio of 1:1.

Results and Discussion

As mentioned above, we used only calcium dioxide along the pre-treatment. This fact, combined with the use of more diluted reagents in the detoxification, made it possible to obtain $65 \pm 10\%$ grams of calcium sulfate (average value of five determinations) instead of 100-110 grams of the sulfate mixture obtained by using the original method, under the cited conditions.

This by-product decrease in itself seems to have a significant effect on the final yield of the reducing sugars into the hydrolysis solution. In fact, by testing this solution with the spectrophotometric method cited above, we found an average quantity of 125 mg, compared with the 88 mg of the original method.

Different Performed Hydrolysis Times

Hydrolysis times exceeding one hour do not seem to influence significantly the reaction yield. A small increase of about 1% yield after two hours and also a decrease for longer time duration were observed. Therefore, even for cost reasons, the better way seems to reach a hydrolysis time of 60 minutes as in the original method.

Recovery From the By-product

Another important point for yield enhancing seems to be the recovery of the fraction of the reducing sugars, co-adsorbed in the by-product mass. For this purpose, we tested the use of the methanol as the recovery solvent. This is preferable to other solvents because of its affinity for sugars and poor solubility for the calcium sulfate. Results of four different sequential extractions with methanol gave indications of the possible recovery of another 20% on average of reducing sugars from the by-product.

Cost Savings

Table 1 shows the main operational and instrumental costs for cellulose hydrolysis process. If only these data are taken into account, the process formerly studied appears to be slightly less expensive than the currently used process, but if the cost per obtained reducing sugars is considered, the last process becomes more profitable: The values are approximatively 0.11 €/mg sugars versus 0.15 €/mg sugars.

In addition, a radical change in the amount of cost savings appears, of course, by considering the fact that by-product (calcium sulfate) revenue should be deducted from the costs. The commercial value for calcium sulfate, as pure reactive, is approximately 79.9 €/kg and the amount obtained was 65 g with a consequent revenue of 5.19 €.

Table 1

The Operational and Instrumental Costs

Substance	A	B
H ₂ SO ₄ (> 96% purity)	Cost: 11.45 €/l Quantity: 55 ml Total cost: 0.63 €	Cost: 11.45 €/l Quantity: 50 ml Total cost: 0.57 €
CaOH ₂ (> 96% purity)	Cost: 17 €/kg Quantity: 7.41 g Total cost: 1.26 €	Cost: 17 €/kg Quantity: 64 g Total cost: 10.88 €
NaOH	Cost: 13.86 €/kg Quantity: 70 g Total cost: 9.70 €	Not used
CH ₃ OH (> 99.8% purity)	Not used	Cost: 14.47 €/l Quantity: 40 ml Total cost: 0.58 €
Energy*		
Heating mantle (used for 3h)	Cost: 0.20 €/kWh kWh:4.5 Total cost: 0.90 €	Cost: 0.20 €/kWh kWh:4.5 Total cost: 0.90 €
Rotavapor (used for 1h):	Cost: 0.20 €/kWh kWh:2 Total cost: 0.40 €	Cost: 0.20 €/kWh kWh:2 Total cost: 0.40 €
Total cost	12.89 €	13.33 €
Unit cost	0.15 €/mg sugars	0.11 €/mg sugars

Notes. A = Previous study; B = Current study. *: Average electricity price for industrial consumers in Italy was considered, all taxes and levies included (Eurostat, 2015).

Conclusions

Significant progresses have been obtained with respect to the original method:

- (1) By using the only calcium hydroxide during the different phases of the process, it seems to be possible to obtain a smaller but more pure quantity of by-product (calcium sulphate instead of a mixture of calcium and sodium sulphate) of higher economic value;
- (2) By reducing the reagents concentration during the detoxification phase;
- (3) By washing the hydrolysis by-product (the calcium sulphate salt) with different solvents, particularly methanol.

Under these conditions, it appears possible to enhance the yield in reducing sugars from 88 to about 125 mg per 2 g of dry matter that correspond to a progress of more than 40% yield. Further investigations are still in progress to improve the yields.

Finally, thanks to all these improvements in the method, it has been possible to achieve notable cost savings with respect to the previously used process. Indeed, the product (reducing sugars) cost moved from 0.15 €/mg to 0.07 €/mg, according to the deduction for by-product revenue.

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